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Claims

- 1. A method of sequencing and distinguishing between nucleic acid sequences on an array, which sequences originate from different sources, which method comprises the steps of,
- a) immobilising target nucleic acid sequences from different sources to said array via a capture moiety comprising a functionality capable of effecting

 10 immobilisation of said target nucleic acid sequences to said array thereby producing immobilised molecules, each immobilised molecule comprising a target nucleic acid sequence and a nucleic acid sequence tag characteristic of the target nucleic acid sequence source and,
- b) sequencing said immobilised molecules whereupon said sequencing identifies a sequence of each of the nucleic acid molecules comprising the characteristic nucleic acid sequence tag to identify the corresponding source of the target nucleic acid sequence.

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- 2. A method according to claim 1 wherein said capture moiety comprises said nucleic acid sequence tag.
- A method according to claim 1 or claim 2 wherein said
 capture moiety comprises a double stranded nucleic acid
 anchoring molecule.
 - 4. A method according to any preceding claim wherein said capture moiety comprises a hairpin oligonucleotide.

- 5. A method according to any preceding claim wherein said target nucleic acid sequence comprises a single stranded DNA polynucleotide.
- 5 6. A method according to claim 3 and claim 5 wherein said DNA polynucleotide is ligated to the 5' end of one strand of said double stranded nucleic acid anchoring molecule that is not used for said anchoring.
- 7. A method according to claim 4 and claim 5 wherein said target nucleic acid sequence comprises a single stranded DNA polynucleotide and said DNA polynucleotide is ligated to the 5' end of said hairpin oligonucleotide.
- 8. A method according to any one of claims 3 to 7 wherein said double stranded anchoring molecule or hairpin comprises a 5' overhanging sequence and the nucleic acid sequence tag is located on said overhanging sequence.
- 9. A method according to claim 7 wherein said capture moiety is a hairpin oligonucleotide comprising a single stranded nucleic acid sequence which contains a region of internal self complementarity, said region being capable of forming a intramolecular duplex comprising the 5' and 3' ends thereof.
- 10. A method according to claim 9 wherein said hairpin oligonucleotide comprises said nucleic acid sequence tag characteristic of said target nucleic acid sequence source positioned immediately adjacent the 5' end of said hairpin and the complement of said nucleic acid sequence tag positioned immediately adjacent the 3' end of said hairpin.

11. A method according to claim 10 wherein said target nucleic acid is ligated to said hairpin oligonucleotide by removing any phosphate groups from 5' and 3' ends of said target nucleic acid whilst providing a single phosphate group at the 5' end of said hairpin oligonucleotide, and incubating said target nucleic acid and hairpin oligonucleotide in the presence of a ligation reagent, whereby a 3' end of the target nucleic acid is ligated to the 5' end of said hairpin oligonucleotide.

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- 12. A method according to any of claims 8 to 11 wherein said overhanging sequence is generated in a cleavage step comprising cleavage of the 3' strand of said double stranded anchoring molecule or hairpin at a cleavage position upstream (5') of or adjacent to the complement of the nucleic acid tag sequence, thereby removing the complement of said tag sequence on said 3' strand.
- 20 13. A method according to claim 12 wherein cleavage is carried out by providing on said double stranded anchoring molecule or hairpin a recognition sequence for an endonuclease capable of cleaving the 3' strand of the anchoring molecule or hairpin at a cleavage site upstream of or adjacent to the complement of nucleic acid tag sequence to remove the complement of said tag sequence on said 3' strand and contacting with said endonuclease.
- 14. A method according to claim 12 or 13 wherein said
 30 cleavage step to generate the overhanging sequence is
 carried out prior to said sequencing and sequencing
 comprises adding one or more nucleotides simultaneously or

sequentially to the 3' hydroxyl group generated by cleavage of the 3' strand and determining the identity of one or more of the added nucleotides.

- 5 15. A method according to claim 12 or claim 13 wherein sequencing of a portion of the target nucleic acid sequence is carried out prior to said cleavage step, said sequencing comprising adding one or more nucleotides simultaneously or sequentially to the 3' end of the anchor molecule or hairpin and determining the identity of one or more of the added nucleotides and sequencing of the nucleic acid sequence tag is carried out after said cleavage step said sequencing comprising adding one or more nucleotides simultaneously or sequentially to the 3' hydroxyl group generated by cleavage of the 3' strand and determining the identity of one or more of the added nucleotides.
- 16. A method according to any preceding claim wherein said nucleic acid sequences on said array are disposed at a20 density such that they are capable of individual resolution using optical microscopy.
 - 17. A method according to claim 16 wherein said immobilised molecules are present on said array at a density of one immobilised molecule per 250nm².

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18. A method according to any preceding claim wherein said method comprises immobilising on said array a first set of nucleic acid sequences isolated from a first source via a capture moiety comprising a characteristic nucleic acid sequence tag and repeating for second and subsequent nucleic acid molecules from second and subsequent sources using

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second and subsequent capture moieties having characteristic nucleic acid sequence tags for each of said sources.

- 19. A method according to claim 1 wherein each of said

 5 immobilised molecules comprises a target nucleic acid
 sequence flanked by first and second adaptor molecules,
 wherein the first adaptor molecule is attached to the 5' end
 of the target nucleic acid sequence and the second adaptor
 molecule is attached to the 3' end of the target nucleic
 10 acid sequence, and the second adaptor includes a nucleic
 acid sequence tag.
- 20. A method according to claim 1 wherein the first adaptor molecules comprise a first amplification primer sequence and the second adaptor molecules comprise a second amplification primer sequence.
- 21. A method according to claim 20 wherein the second adaptor molecule further comprises a sequencing primer20 binding sequence positioned between the amplification primer sequence and the nucleic acid sequence tag.
 - 22. A method according to claim 20 wherein the amplification primer sequence in the second adaptor molecule also functions as a sequencing primer binding sequence.
 - 23. A method according to any one of claims 19 to 22 wherein in step a) immobilised molecules are produced by solid-phase amplification on said array.

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24. A method according to claim 23 wherein said solid-phase amplification comprises the following steps:

- i) providing template nucleic acid constructs, each template construct comprising a target nucleic acid sequence and two adaptor molecules, wherein one adaptor molecule is attached to the 5' end of the target nucleic acid sequence and the other adaptor molecule is attached to the 3' end of the target nucleic acid sequence, wherein at least one of the adaptors includes a nucleic acid sequence tag; ii) providing a solid support having immobilised thereon amplification primer molecules capable of directing 10 amplification of the template nucleic acid constructs via interaction with amplification primer sequences in the adaptor molecules; and iii) performing a nucleic acid amplification reaction using the template nucleic acid constructs and the immobilised 15 amplification primer molecules, thereby forming a plurality of immobilised amplification products each of which comprises a target nucleic acid sequence and a nucleic acid sequence tag.
- 25. A method according to claim 24 wherein the amplification primer molecules of step ii) comprise a mixture of first primer molecules complementary to an amplification primer sequence in one of the adaptors and second primer molecules corresponding to an amplification primer sequence in the other adaptor.
 - 26. A method according to claim 25 wherein in a first step of the amplification reaction the immobilised primers are contacted with template constructs to be amplified under conditions which permit specific binding of one of the immobilised primers to a complementary amplification primer sequence present in one of the adaptor molecules.

- 27. A method according to claim 24 or claim 25 wherein the template nucleic acid constructs are also immobilised on the solid support via a functionality at the 5' end of an adaptor molecule prior to the nucleic acid amplification reaction.
- 28. A method according to any one of claims 24 to 27 wherein the template nucleic acid constructs provided in step i) comprise two or more sets of constructs, each set of constructs comprising nucleic acid sequences isolated from a different source, wherein each set of constructs comprises a different nucleic acid sequence tag characteristic of the source of the target nucleic acid sequences.

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- 29. A method according to any one of the preceding claims wherein the nucleic acid sequence tag is from 1 to 10 nucleotides in length.
- 20 30. A method according to claim 29 wherein the nucleic acid sequence tag is 4, 5 or 6 nucleotides in length.
- 31. A method according to any of the preceding claims wherein said sequencing comprises at least one cycle of sequencing and is performed in the presence of a polymerase, said sequencing comprising addition of one or more nucleotides simultaneously or sequentially wherein each nucleotide comprises a characteristic label, and a blocking group that is capable of preventing uncontrolled
- 30 polymerisation, wherein a cycle of sequencing comprises identifying any incorporated nucleotide incorporated by said polymerase and removing the blocking group and

characteristic label from said nucleotide incorporated by said polymerase.